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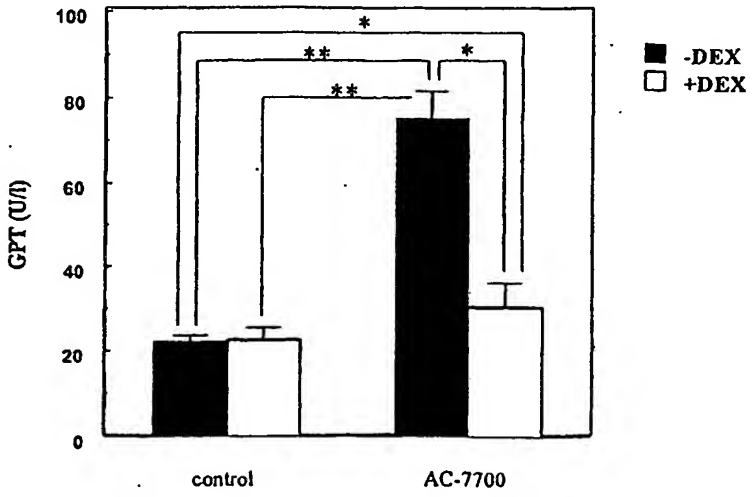
(54) ANTITUMOR AGENTS

(57) An anti-tumor agent comprises a tubulin polymerization-inhibitory active substance having anti-tumor activity and an anti-inflammatory active substance (in combination; in combined use) is made. Both the effective components can be contained in a single pharmaceutical,or can be combined in the form of separately administered 2 kinds of pharmaceuticals.

In the use of a tubulin polymerization-inhibitory active substance as an effective component in an anti-tumor agent, the anti-tumor agent can maintain its pharmaceutically effective dosage, but at the same time can significantly increase the lethal dosage and improve toxicity at the pharmaceutically effective dosage of the tubulin polymerization inhibitory active substance.

Fig.1



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Description

Field of the Invention

[0001] The present invention relates to a novel anti-tumor agent, and more particularly it relates to an anti-tumor agent as a combination of a tubulin-polymerization inhibiting active substance having anti-tumor activity with an anti-inflammatory active substance. Since the use of an anti-tumor medical agent (anti-tumor agent) containing a tubulin polymerization-inhibitory active substance in combination with an anti-inflammatory active substance can significantly increase the lethal administration dosage of a tubulin polymerization-inhibitory active substance and at the same time can maintain its pharmaceutically effective dosage at a level substantially comparable to that for non-combined administration, the safety zone for the medical agent can be expanded. Also, the toxicity of the tubulin polymerization-inhibitory substance at the pharmaceutically effective dosage can be significantly improved at the same time. As a result, the usefulness as an anti-tumor agent to be administered by medical doctors, et al can be remarkably expanded, and the burden to patients et al can be reduced.

[0002] According to the present invention, an anti-tumor pharmaceutical (pharmaceutical preparation) containing a tubulin polymerization-inhibitory active substance having anti-tumor activity to be used in combination with an anti-inflammatory active substance, and a toxicity reducing agent to be used for an anti-tumor pharmaceutical containing the tubulin polymerization-inhibitory active substance, wherein the agent comprises an anti-inflammatory active substance, are also provided.

[0003] Further, the present invention also relates to a method for the anti-tumor, wherein the method includes methods for a medical treatment and an improvement of tumors, a prevention of progression of tumor, and a prevention of tumor; to uses of the above-mentioned 2 effective components for a medical product such as an anti-tumor agent; and to a combination of the above-mentioned 2 effective components wherein the two components are used as a medical product such as an anti-tumor agent, simultaneously or separately, etc.

Background Art

[0004] One of anti-tumor agents expected for their development relates to the development of medical agents (anti-tumor agents), now under way, which contain a tubulin polymerization-inhibitory active substance as an effective component. (Refer to Biochem. Mol. Biol. Int. 25 (6), 1153-1159 (1995); Br. J. Cancer 71 (4), 705-711 (1995); J. Med. Chem. 34 (8), 2579-2588 (1991); Biochemistry 28 (17), 6904-6991 (1989); US Patent No. 5,561,122; Japanese Laid-Open Patent Application JP-A-07-228,558(1995); Japanese Laid-Open Patent Application JP-A-08-301,831(1996); etc.).

[0005] As a result of detailed studies about the medical agent containing a tubulin polymerization-inhibitory active substance, the present inventors have predicted, among others, the possibility of expanding the utility of this medical agent by maintaining the pharmaceutically effective dosage, increasing the lethal dosage and improving the toxicity at the pharmaceutically effective dosage, because the administration of this medical agent has been restricted in respect to the safety zone (a ratio between the lethal administration dosage and the pharmaceutically effective dosage) and the toxicity at the pharmaceutically effective dosage, and thus have been engaged in research and examination for the creation of a medical agent by maintaining the pharmaceutically effective dosage of the effective component in the anti-tumor agent and simultaneously increasing the lethal dosage so that the safety zone for its administration can be expanded, and the toxicity level at the pharmaceutical effective dosage can be reduced, in order to expand its utility as a medical agent, and also in order to reduce burdened to patients et al.

[0006] Under such situations, the development of an anti-tumor agent which retains the effectiveness (medical effect) of an anti-tumor agent containing a tubulin polymerization-inhibitory active substance as an effective component but which is improved only on the toxicity has been desired.

Problem to be Solved by the Invention

[0007] The problem to be solved according to the present invention is, when a tubulin polymerization-inhibitory active substance is used as an effective component in an anti-tumor agent, to develop a medical agent (anti-tumor agent) which can maintain its pharmaceutically effective dosage, but at the same time can significantly increase on the lethal dosage and improve toxicity at the pharmaceutically effective dosage of the tubulin polymerization inhibitory active substance.

Solution for Problem

[0008] In order to solve the problem, the present inventors have mainly examined combretastatins, stilbenes, derivatives thereof, etc. as a tubulin polymerization-inhibitory active substance which can be utilized as an effective com-

ponent in such an anti-tumor agent. The inventors have made intensive research works to find out a possible method for retaining the pharmaceutically effective dosage while increasing the lethal dosage, and also for improving various kinds of toxicity caused by administering the pharmaceutically effective dosage, particularly gastroinestinal toxicity, hepatic toxicity and cardiovascular toxicity. These works have resulted in a finding that the combined use of an anti-inflammatory active substance, particularly an anti-inflammatory active steroid substance can significantly increase the lethal administration dosage of the tubulin polymerization-inhibitory active substance, favorably to approximately double the original lethal dosage, and can significantly lower toxicity, particularly, its gastroinestinal toxicity, hepatic toxicity and cardiovascular toxicity, while the pharmaceutically effective dosage can be retained substantially at the same level to that without the combined use, and thus, its safety zone as a medical agent can be remarkably expanded and the toxicity can be significantly improved within the pharmaceutically effective dosage range, the present invention eventually having been completed based on various knowledge including this finding.

[0009] Namely, the present invention relates to an anti-tumor agent comprising at least a tubulin polymerization-inhibitory active substance having anti-tumor activity and at least an anti-inflammatory active substance (which covers the combination of both the substances) [an anti-tumor agent according to the present invention].

[0010] The anti-tumor agent according to the present invention may be in the form of a pharmaceutical (pharmaceutical preparation) containing at least a tubulin polymerization-inhibitory active substance and an anti-inflammatory active substance in a single pharmaceutical (pharmaceutical preparation), or may be in the form of a set of 2 pharmaceuticals (pharmaceutical preparations), for example; an anti-inflammatory agent and an anti-tumor pharmaceutical (pharmaceutical preparation) containing the tubulin polymerization-inhibitory active substance, or in the form of different pharmaceuticals (pharmaceutical preparations) wherein 2 kinds of the pharmaceuticals (pharmaceutical preparations) are used in combination.

[0011] The tubulin polymerization-inhibitory active substance may be any substance as far as it has tubulin polymerization-inhibitory activity, and there is no other particular restriction. It is necessary to select a substance which has anti-tumor activity, but any such a substance may be adopted without restriction, either it is a known substance or a substance to be developed in future. For example, such a tubulin polymerization-inhibitory active substance may be selected from the group consisting of combretastatines and derivatives thereof, vinca alkaloids such as vinblastine, etc. and derivatives thereof, colchicines and derivatives thereof, dolastatins and derivatives thereof, podophyllotoxins and derivatives thereof, steganacins and derivatives thereof, amphethiniles and derivatives thereof, flavonoids and derivatives thereof, rhizoxins and derivatives thereof, curacins A and derivatives thereof, epothilones A and derivatives thereof, epothilones B and derivatives thereof, welwistatins and derivatives thereof, phenstatins and derivatives thereof, 2-strylquinazoline-4(3H)-ones and derivatives thereof, stilbenes and derivatives thereof, 2-aryl-1,8-naphthyridin-4(1H)-ones and derivatives thereof, 5,6-dihydroindolo(2,1-a) isoquinolines and derivatives thereof, 2,3-benzo(b)thiophenes and derivatives thereof, 2,3-substituted benzo(b)furans and derivatives thereof, 2,3-substituted indoles and derivatives thereof, and 2-methoxyestradiol (refer to the WO 00/48606 Specification).

[0012] Various reports have been made on combretastines, stilbenes and derivatives thereof having anti-tumor activity (refer to J. Med. Chem. 41: 3022-3032 (1998); Bioorg. Med. Chem. Lett. 8:3153-3158 (1998); Bioorg. Med. Chem. Lett. 8:3371-3374 (1998); US Patent No. 5,561,122; US Patent No. 5,430,062; Japanese Laid-Open Patent Application JP-A-07-228,558(1995); Japanese Laid-Open Patent Application JP-A-08-301,831(1996), the WO93/23,357 specification, the WO99/51,246 specification; etc.), and those substances described therein call be utilize according to the present invention (and all the descriptions about these tubulin polymerization-inhibitory active substances are included in the present specification by reference). As a typical example of the derivatives, (Z)-N-[2-methoxy-5-[2-(3,4,5-trimethoxyphenyl)vinyl]phenyl]-L-serinamide hydrochloride (hereinafter called "AC-7700) may be cited.

[0013] In addition, all the substances cited in the WO 00/48,606 specification as to have tubulin polymerization-inhibitory activity can be used as a tubulin polymerization-inhibitory active substance according to the present invention, and all the substances cited to have tubulin polymerization-inhibitory activity, any descriptions about these substances in the international patent publication (specification) or in prior art publications cited therein (as related descriptions) are included (incorporated) in the present specification by reference.

[0014] No particular restriction exists on the anti-inflammatory active substance to be used according to the present invention, but it can be favorably selected from the group consisting of anti-inflammatory active steroid substances and analogous substances thereof, anti-inflammatory active non-steroid substances and analogous compounds thereof, and anti-inflammatory or immuno-suppressive active substances.

[0015] The anti-inflammatory active substance to be used according to the present invention may be preferably an anti-inflammatory active steroid substance.

[0016] Such an anti-inflammatory active substance may be selected from the group consisting of Dexamethasone and derivatives thereof, prednisolone and derivatives thereof, methylprednisolone and derivatives thereof, betamethasone and derivatives thereof, triamcinolone and derivatives thereof, paramethasone and derivatives thereof, beclomethasone and derivatives thereof, flucinolone acetonide and derivatives thereof and cortisol (natural glucocorticoid) and derivatives thereof.

[0017] It can be favorably selected from the group consisting of Dexamethasone and derivatives thereof, particularly Dexamethasone, phosphate ester thereof and salts thereof (such as sodium salt, etc.). Conveniently, a commercial distributed anti-inflammatory agent can be procured for the use on the market.

[0018] As typical examples for the 2 kinds of the effective components, the tubulin polymerization-inhibitory active substance may be selected particularly from the group consisting of combretastatins and derivatives thereof and stilbenes and derivatives thereof, and the anti-inflammatory active substance may be also selected from the group consisting of Dexamethasone and derivatives thereof (ester, etc.).

[0019] A pharmaceutical (pharmaceutical preparation) containing the tubulin polymerization-inhibitory active substance (an anti-tumor pharmaceutical (pharmaceutical preparation)) and a pharmaceutical (pharmaceutical preparation) containing an anti-inflammatory active substance (an anti-inflammatory agent) can be simultaneously administered or can be administered at different times. Accordingly, [these pharmaceuticals (pharmaceutical preparations)] may be in the form for respective administrations or in the form of a single pharmaceutical (pharmaceutical preparation). Both the pharmaceuticals may be in different forms for respective administration, and in this case, both the pharmaceuticals have to constitute respectively different pharmaceuticals.

[0020] The tubulin polymerization-inhibitory active substance can be used in the form of an anti-tumor pharmaceutical (pharmaceutical preparation; pharmaceutical agent) and the anti-inflammatory active substance can be used in the form of an anti-inflammatory agent.

[0021] According to another mode, the present invention relates to an anti-tumor pharmaceutical (pharmaceutical preparation; pharmaceutical agent) containing a tubulin polymerization-inhibitory active substance having anti-tumor activity, wherein the anti-tumor pharmaceutical is to be used in combination with or as a set with an anti-inflammatory active substance (an anti-tumor pharmaceutical (pharmaceutical preparation; pharmaceutical agent) according to the present invention).

[0022] According to further another mode, the present invention relates to a toxicity-reducing agent to be used for an anti-tumor pharmaceutical (pharmaceutical preparation; pharmaceutical agent) containing a tubulin polymerization-inhibitory active substance, wherein the toxicity-reducing agent comprises an anti-inflammatory active substance (a toxicity-reducing agent according to the present invention).

[0023] The present invention covers first of all the anti-tumor agent according to the present invention, but it additionally covers the 2 different modes of inventions. Those agents according to the 2 different modes of invention are substantially same with the anti-tumor agent according to the present invention in a sense that both the 2 different modes of invention are related to a medical agent wherein 2 kinds of effective components: a tubulin polymerization-inhibitory active substance having anti-tumor activity and an anti-inflammatory active substance are combined, and therefore, can be easily worked based on explanations about the anti-tumor agent according to the present invention. The term of "an anti-tumor pharmaceutical (pharmaceutical preparation; pharmaceutical agent) " is a term used for its differentiation from the "anti-tumor agent" according to the present invention, and any and all medical agents containing the tubulin polymerization-inhibitory active substance and used for the therapy, betterment or other treatments of tumors are equally and completely covered under the term, regardless of whatever names those medical agents are termed, including anti-tumor agents, etc.

[0024] According to the present invention, an anti-tumor medical agent can be, for example, a combination of the 2 kinds of the effective components in respectively different pharmaceutical forms, and moreover in the forms which can be administered at the same time or different times. Also, these 2 effective components may be included in the same pharmaceutical (pharmaceutical preparation; pharmaceutical agent) which allows individual administration by pharmaceutical units. Even in such modes of applications, medical agents containing at least the 2 kinds of the effective components are also covered by the anti-tumor agent according to the present invention.

[0025] Consequently, it is not necessary that plural pharmaceuticals respectively containing at least the 2 kinds of the effective components to be used according to the present invention are jointly and fixedly contained in a specific package or container, and even when these pharmaceuticals are respectively and independently present, these pharmaceuticals shall be covered by the anti-tumor agent according to the present invention as far as these pharmaceuticals are used for the same purpose as according to the present invention (the realization of an anti-tumor effect).

[0026] Furthermore, although it is indispensable that the anti-tumor agent according to the present invention contains the 2 kinds of the effective components which are used in an appropriate combination, further different effective components [components having the same medical effect (anti-tumor components) or components having a different medical effect, components for enhancing an intended medical effect, components for the further reduction of toxicity (a side effect), and other components] may be used for combination or inclusion in pharmaceuticals according to the present invention, as far as these additional components do not hinder the effect of the present invention. In the preparation of pharmaceuticals, additive components required can be appropriately selected and used for the pharmaceutical preparation.

[0027] According to another mode, the present invention relates to a method for the anti-tumor, which comprises administering containing a tubulin polymerization-inhibitory active substance having anti-tumor activity and an anti-

inflammatory active substance to a living subject. The method includes methods for a medical treatment and an improvement of tumors, a prevention of progression of tumor, and a prevention of tumor. These 2 kinds of effective components can be administered to a living body at the same time, or separately at different times. The administration form as such can be selected from various forms in the above-mentioned anti-tumor agent according to the present invention, the anti-tumor pharmaceutical preparation, and the toxicity-reducing agent.

[0028] According to further another mode, the present invention relates to uses of a tubulin polymerization-inhibitory active substance having anti-tumor activity and an anti-inflammatory active substance for a medical product such as an anti-tumor agent. The tubulin polymerization-inhibitory active substance having anti-tumor activity and the anti-inflammatory active substance are can be used individually in the different pharmaceutical preparation forms. The form for uses in the medical product as such can be selected from various forms in the above-mentioned anti-tumor agent according to the present invention, the anti-tumor pharmaceutical preparation (agent), and the toxicity-reducing agent.

[0029] According to further another mode, the present invention relates to a combination of a tubulin polymerization-inhibitory active substance having anti-tumor activity with an anti-inflammatory active substance wherein the two substances are used as a medical product such as an anti-tumor agent, simultaneously or separately.

Brief Explanation of Drawings

[Figure 1]

[0030] Figure 1 shows results from the toxicity test with tumor-bearing rats in Example 1 (Scheffe's F test; *p<0.05, **p<0.01)
F344 rats subcutaneously transplanted MT-9 tumor / Dexamethasone (1mg/kg)/AC-7700 (10mg/kg); Blood biochemical indices: GPT; ■-DEX; □+DEX.

[Figure 2]

[0031] Figure 2 shows results from the toxicity test with tumor-bearing rats in Example 1 (Scheffe's F test; *p<0.05). F344 rats subcutaneously transplanted MT-9 tumor / Dexamethasone (1mg/kg)/AC-7700 (10mg/kg); Blood biochemical indices: CPK; ■:-DEX; □:+DEX.

Mode of Working the Invention

[0032] In the following, the present invention shall be explained mainly on the mode as an anti-tumor agent according to the present invention, but other modes of the present invention can be similarly understood based on this explanation because the same 2 kinds of medical components: a tubulin polymerization-inhibitory active substance having anti-tumor activity and an anti-inflammatory active substance are combined in all these modes for working the present invention.

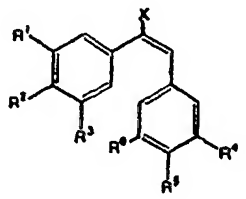
[0033] The anti-tumor agent according to the present invention is a medical agent which contains at least said 2 kinds of effective components, plural components being capable to be administered at the same or different times, the 2 kinds of components being administered in the form of a same pharmaceutical or different pharmaceuticals for combined use for the broad purpose of tumor suppression, such as the betterment, prevention, therapy, growth inhibition, etc. of tumors in mammals, particularly human. As described above, as far as this effect can be achieved, it can additionally contain or be combined with other medical components, and it can also contain other components which are required for pharmaceutical preparation.

[0034] As explained above, the tubulin polymerization-inhibitory active substance to be used according to the present invention may be selected from known tubulin polymerization-inhibitory active substances having anti-tumor activity (refer to the above cited prior art publications), and the below described stilbene derivatives can be cited as particularly preferable ones.

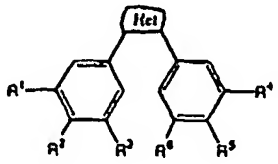
[0035] Examples of the stilbene derivatives to be used according to the present invention may include compounds having the fundamental skeleton of cis-stilbene, which exhibit the in-vitro activities of tubulin polymerization inhibition and anti-tumor. As for anti-tumor activity, those compounds particularly exhibiting the activity of tumor cell growth inhibition are preferable. Any of the stilbene derivatives, either known or to be found in future, should be included in the stilbene derivatives to be used according to the present invention. Moreover, the stilbene derivatives shall include derivatives which are converted to a stilbene derivative in an animal body. As far as a stilbene derivative can exhibit the intended anti-tumor activity according to the present invention when used in an animal body, and may it be a salt, ester, solvate such as hydrate, etc., as far as it is a pharmaceutically acceptable derivative, it can be used as a stilbene derivative having tubulin polymerization-inhibitory activity according to the present invention.

[0036] Typical stilbene derivatives having the fundamental skeleton of cis-stilbene include compounds preferably

represented by the following formulae (1) and (2). These compounds include those in the form of various salts, hydrates and solvates, and particularly those in pharmaceutically acceptable forms.



(1)



(2)

wherein R^1, R^2 and R^3 respectively and independently denote a lower alkoxy group, R^4, R^5 and R^6 respectively and independently denote a hydrogen atom, a halogen atom (fluorine, chlorine, etc.), any of substitution groups including nitro, hydroxy, lower alkoxy, phosphate ester (a substitution group formed by phosphoric ester formation with a hydroxy group: $-OP_3H_2$, hereunder meaning the same), phosphate amide (a substitution group formed by phosphoric amide formation with an amino group: $-NHPO_3H_2$, hereunder meaning the same), amino lower alkoxy, lower alkyl amino lower alkoxy, di-lowera alkyl amino lower alkoxy, mercapto, lower alkyl thio, amino, lower alkyl amino, di-lower alkyl amino, lower alkyl, amino lower alkyl, trifluoromethyl, lower alkanoyl, lower alkanoyl amino and amino acid acyl amino, X denotes a hydrogen atom or a nitrile group, and Het denotes a heterocycle.

[0037] The lower alkyl and lower alkoxy groups are respectively to have 1 ~5 carbon atoms. Also, the lower alkanoyl group is to have 2~6 carbon atoms.

[0038] An amino acid acyl group in the amino acid acyl amino group is an acyl group derived from an amino acid, and examples of the amino acid may include α-amino acid, β-amino acid and γ-amino acid. Preferable examples of the amino acid may include glycine, alanine, leucine, serine, lysine, glutamic acid, aspartic acid, threonine, valine, isoleucine, ornithine, glutamine, asparagine, tyrosine, phenyl alanine, cystine, methionine, alginine, β-alanine, tryptophan, proline, histidine, etc. Particularly preferable in respect of the pharmaceutical effect and safety are threonine and serine. These amino acids may be of any of L-, D- and DL-forms, but the L-form is preferable.

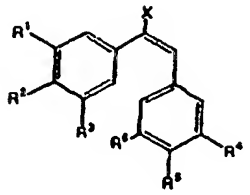
[0039] Examples of the heterocycle may include a tetrazole ring, a thiazole ring, etc. When the heterocycle is a thiazole ring, it may be substituted. Examples of a substitution group in this case may include lower alkyl, amino, mono-lower alkyl amino, di-lower alkyl amino, hydrazino, halogen atom (fluorine, chlorine, etc.) and lower alkoxy. Incidentally, the lower alkyl and lower alkoxy groups are respectively to have 1~5 carbon atoms.

[0040] As described above, a stilbene derivative to be used according to the present invention is structurally a compound having a cis-stilbene skeleton, and a compound exhibiting tubulin polymerization-inhibitory activity and antitumor activity. As a specific example of the stilbene derivative, combretastatin-A4 may be cited, but the stilbene derivative should not be particularly restricted, and may include any stilbene derivatives capable of inhibiting tumor growth, which have been disclosed in prior art publications, for example, patent gazettes, etc. (refer to US Patent Nos. 4,996,237; 5,561,122 and 5,430,062; and Japanese Laid-Open Patent Applications JP-A-07-228,558(1995); JP-A-08-301,831(1996) and JP-A-10-81,673(1998); etc.). Stilbene derivatives described in these prior art publications can be utilized as stilbene derivatives according to the present invention as far as those are covered under the above stated definitions, and as described above, those disclosures in the prior art publications are included as a part constituting the present patent specification.

[0041] When the stilbene derivatives are to be manufactured, these compounds can be manufactured according to known technology including manufacturing processes disclosed in the above described publications. Stilbene derivatives to be developed in future may be also manufactured and utilized in the same manner as described above.

[0042] Stilbene derivatives to be used according to the present invention may include those in the form of salts, esters, solvates such as hydrates and other derivatives, as well as derivatives which may be converted in animal body to stilbene derivatives as far as those derivatives can exhibit said activity in animal body.

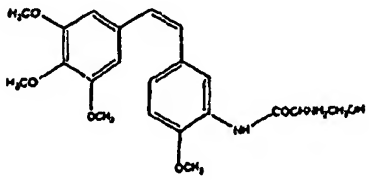
[0043] More preferable stilbene derivatives to be used according to the present invention are represented by the following formula (1'):



(1')

[0044] In the formula (1'), R^1, R^2, R^3 and R^5 respectively denote a methoxy group, R^4 denotes either an amino group or an amino acid acyl amino group, and R^6 and X respectively denote a hydrogen atom.

[0045] Among compounds represented by the formula (1'), a compound represented by the formula (3) [(Z)-N-[2-methoxy-5-[2-(3,4,5-trimethoxyphenyl) vinyl] phenyl]-L-serina mide] is particularly preferable [which is hereunder called Compound (3)]. The Compound (3) may be in the form of a salt, which may include hydrochloride (AC-7700), acetate, methane sulfonate, etc.



(3)

[0046] The manufacture of Compound (3) (including pharmaceutically acceptable salts, hydrates and solvates thereof) as well as the manufacture of orally or non-orally administered pharmaceutical compositions containing Compound (3) and an inert, pharmaceutically acceptable carriers or diluent have been disclosed in Japanese Laid-Open Patent Application JP-A-08-301,831(1996) in a broad range, which can be referred to for the manufacture thereof.

[0047] The compound can be used, as a medical agent according to the present invention, in the form of a pharmaceutical (pharmaceutical preparation) which contains a tubulin polymerization-inhibitory active substance having anti-tumor activity. In this case, it can be used in the form of a single pharmaceutical (pharmaceutical preparation) containing the tubulin polymerization-inhibitory active substance as a (major) effective component, or it can be also used in the form of a pharmaceutical (pharmaceutical preparation) which combines the anti-inflammatory active substance with this component (a pharmaceutical combining 2 kinds of effective components). There is no particular restriction on the form of pharmaceutical preparations in this case. It can be used in the form of an orally administered pharmaceutical or a non-orally administered pharmaceutical, particularly in the form of an injection. Investigations for their use as an anti-tumor agent have been already under way on some of the tubulin polymerization-inhibitory active substances (for example, combretastatines, vinca alkaloids, colchicinoids, dolastatins, podophyllotoxins, rhizoxins, 2-methoxyestradiol, etc.), and thus, their pharmaceuticals (pharmaceutical preparations) can be easily prepared based on public knowledge obtained in relation to such investigations.

[0048] An anti-inflammatory active substance to be used in the anti-tumor agent according to the present invention can be combined and used in said pharmaceuticals, but what has been known and used as an anti-inflammatory agent ca be separately used for the combined use with a pharmaceutical which contains the tubulin polymerization-inhibitory active substance having anti-tumor activity. It can be used according to a known administration method, etc. for an anti-inflammatory agent. Due to a component to be combined for use or the combined use of an anti-inflammatory active substance according to the present invention, the lethal dosage of the tubulin polymerization-inhibitory active substance is increased, preferably to about twice or more, and remarkable improvement on the toxicity at the pharmaceutically effective dosage can be achieved, particularly on gastrointestinal toxicity (betterment of diarrhea, etc.), hepatic toxicity (lowering of GPT, etc.) and cardiovascular toxicity (lowering of CPK, etc.). On the other hand, it has been found that the pharmaceutically effective dosage has not been affected by the presence or absence of the combined use according to the present invention.

[0049] As a result, when a tubulin polymerization-inhibitory active substance is used as an anti-tumor agent, a re-

markable improvement can be achieved on the lethal toxicity and the toxicity at the pharmaceutically effective dosage, so that medical people such as doctors, et al can conveniently use this medical agent, and the burden on patients administered of this medical agent can be greatly reduced, too.

[0050] For example, depending upon symptoms of patients, etc., the dosage of a tubulin polymerization-inhibitory active substance, for example in the case of AC-7700 (an injection), can be preferably about 0.1~10000mg, more preferably about 0.5~1000mg, and further preferably about 1~500mg per patient a day. The dosage of an anti-inflammatory active agent to be used in combination according to the present invention, for example in the case of Dexamethasone sodium phosphate (an injection), can be preferably about 0.1~10000mg, more preferably about 0.5~1000mg and further preferably about 1~500mg per patient a day.

[0051] When these agents are to be orally administered, respective dosages can be within a range of about 2~20 times the dosage used as an injection.

[0052] The dosage for the administration of the 2 kinds of effective components according to the present invention together with other components or as derivatives thereof can be optionally selected in utilization of prior art technology and measures, by referring to the above described ranges of the dosages.

[0053] The 2 kinds of components as indispensable effective components to be used according to the present invention can be respectively contained in different pharmaceutical forms and independently administered to patients who desire an anti-tumor effect, but as described above, these 2 components can contain or be combined with other pharmaceutical components to be used as a medical agent exhibiting an anti-tumor effect, and these cases are also naturally covered by the present invention as far as these medical agents exert an anti-tumor effect. A tubulin polymerization-inhibitory active substance can be combine with an anti-inflammatory active substance for medical uses, and in this case, further components can be contained or be combined with these 2 kinds of components for the use of a tubulin polymerization-inhibitory active substance so that a similar anti-tumor effect can be exerted, and such a use is also covered by the present invention.

[0054] In the preparation of pharmaceuticals, pharmacologically acceptable, various preparatory substances can be also contained (as supplemental agents, etc.). Substances for pharmaceutical preparation can be optionally selected depending upon the form of preparations, examples of which may include vehicles, diluents, additives, disintegrators, binders, coating agents, lubricants, sliding agents, smoothing agents, flavoring agents, sweeteners, solubilizers, etc. Furthermore, specific examples of preparatory substances may include magnesium carbonate, titanium dioxide, lactose, mannitol and other saccharides, talc, milk casein, gelatin, starch, cellulose and derivatives thereof, animal and vegetable oils, polyethylene glycol, and solvents such as sterile water and mono- or polyhydric alcohols, for example, glycerol, etc.

[0055] It is not necessary to include all the effective components to be used according to the present invention in the same single pharmaceutical, and respective components or the 2 components may be appropriately contained in one or two pharmaceuticals. In this case, the components may be prepared to various forms of pharmaceuticals, either known or to be developed in future, for example, including various administration forms such as for oral administration, abdominal administration, dermal administration, inhalation administration, intravenous administration, etc. In order to prepare pharmaceutical components to be used according to the present invention into such various pharmaceutical forms, methods, either known or to be developed in future, may be optionally adopted.

[0056] Examples of such various forms of pharmaceuticals may include appropriate solid or liquid pharmaceutical forms, such as granules, powder, coated tablets, tablets, (micro-)capsules, depositories, syrups, juices, suspensions, emulsions, drops, injection solutions, preparations for extended release of an active substance, etc.

[0057] Medical agents according to the present invention, prepared in pharmaceutical forms such as cited above in the examples, should naturally contain pharmaceutically effective amounts of said components in order to achieve the intended effect.

(Anti-tumor pharmaceutical according to the present invention)

[0058] As described above, an anti-tumor pharmaceutical containing a tubulin polymerization-inhibitory active substance having anti-tumor activity, which is characterized by the combined use with an anti-inflammatory active substance, is covered by the present invention, and since this pharmaceutical is substantially same with the anti-tumor agent according to the present invention in respect to the combined use of the 2 kinds of medical components, a skilled person in the art should be able to practice this invention based on the above described detailed explanation and later described examples, as well as prior art technology.

(Toxicity-reducing agent according to the present invention)

[0059] Similarly, a toxicity-reducing agent for an anti-tumor pharmaceutical containing a tubulin polymerization-inhibitory active substance, wherein an anti-inflammatory active substrate is contained, is also covered by the present

invention, and since this toxicity-reducing agent is substantially same with the anti-tumor agent according to the present invention in respect to the combined use of the 2 kinds of medical components in the same manner as described above, a skilled person in the art should be able to practice this invention based on the above described detailed explanation and later described examples, as well as prior art technology.

[0060] As described above, according to other modes, the present invention lies in the followings:

i) A method for the anti-tumor, which comprises administering containing a tubulin polymerization-inhibitory active substance having anti-tumor activity and an anti-inflammatory active substance to a living subject, wherein the method includes methods for a medical treatment and an improvement of tumors, a prevention of progression of tumor, and a prevention of tumor;
ii) Uses of a tubulin polymerization-inhibitory active substance having anti-tumor activity and an anti-inflammatory active substance for a medical product such as an anti-tumor agent; and
iii) A combination of a tubulin polymerization-inhibitory active substance having anti-tumor activity with an anti-inflammatory active substance wherein the two substances are used as a medical product such as an anti-tumor agent, simultaneously or separately.

[0061] These modes of the present invention can all be carried out readily on the basis of the descriptions about the above-mentioned anti-tumor agent, anti-tumor pharmaceutical preparation (agent), and/or toxicity-reducing agent according to the present invention or the after described Examples and the like, with reference to known art, if necessary.

Preferred Modes for Operation

[0062] In the following, the present invention shall be explained in reference to illustrative examples, but the present invention should not be restricted by these examples in any manner.

[Example 1]

(1) Tumor cell line and experimental animal

[0063] Rat tumor cell line (transplant rat strain);
Malignant fibrous histiocytoma MT-9 (F344, male)

[0064] MT-9 was obtained as in-vitro cultured cells, which were cultured in RPMI1640 medium containing 10% FBS and then, 10^7 or more of the cells were subcutaneously transplanted to rats on their back. After tumor formation, tumor slices (about 100mg) were inoculated subcutaneously into rats using cannula for serial passage.

[0065] F344 (5 weeks old) was acquired from Charles River Japan.

(2) Medicines and administration method

[0066] As the tubulin polymerization-inhibitory active substance having anti-tumor activity, (Z)-N-[2-methoxy-5-[2-(3,4,5-trimethoxyphenyl)vinyl]phenyl]-L-serinamide hydrochloride (AC-7700) was used.

[0067] AC-7700 had been stored in a dark place at a low temperature (5°C) after the synthesis, and after weighing, it was dissolved in physiological saline immediately before the administration.

[0068] As the Dexamethasone (its derivative), "Decadron S injection" made by Banyu Pharmaceutical (Demethasone sodium phosphate, its chemical term being 16α-methyl-9α-fluoroprednisolone 21-phsophate disodium salt, hereinafter simply called "Dexamethasone" or "DEX") was dilued with physiological saline immediately before the administration and was injected intravenously.

(3) Procedure for the biochemical testing of blood

[0069] Under ether anesthesia, rats were subjected to ventrotomy, and blood was sampled from inferior vena cava using heparin-containing syringes. The blood samples were centrifuged at 3000rpm for 10 minutes to recover plasma, and the GOT, GPT, CPK and LDH concentration in plasma were determined using Fuji Dry Chem. When the measurement was not conducted immediately, the plasma samples had been stored at -80°C until the determination was made.

(4) Procedure for the evaluation of in vivo pharmaceutical effect (anti-tumor effect)

[0070] The MT-9/F344 system: Tumors serially passed by the subcutaneous transplantation were extirpated. After

binding tissues and necrosis portions in the tumors had been removed, tumor tissues were minced with scissors and made pasty, and about 50 ~ 100mg of the tumor tissues were subcutaneously transplanted to F344 rats on their back (day 0).

[0071] After the tumors were multiplied to be measurable (about 1~2 weeks later), the tumor size (tumor volume) and body weight were measured and divided into tumor size-and body weight-matched groups.

[0072] The tumor sizes (tumor volumes) and body weights were measured every day from the next day to about the third day after the administration had been completed. Incidentally, the tumor volume was calculated according to the following formula:

$$\text{Tumor volume } (mm^3) = [(\text{larger diameter, mm}) \times (\text{smaller diameter, mm})]^2 \times 1/2.$$

[0073] For the determination of the anti-tumor effect, the T/C and I.R. values were calculated according to the following formula, and when the T/C value was 50% or less (the I.R. value being 50% or more) and statistically significant difference from the value for control existed, the anti-tumor effect was judged to be positive, and the anti-tumor effect on a day when the maximum anti-tumor effect was observed was determined as the pharmaceutical effect.

$$T/C(\%) = [(\text{tumor volume for the medicine administered group}) + (\text{tumor volume for the control group})] \times 100$$

$$I.R.(\%) = 100 - T/C.$$

[0074] Weight changes were derived by the deduction of the body weight on the day when the treatment was started from the body weight on the day for evaluating the pharmaceutical effect. Incidentally, the tumor weight (g) was calculated by conversion as (tumor volume × 1/1000), and changes in the body weight excluding the tumor weight were determined based on the deduction of respective tumor weights from the body weight.

(5) Statistical analysis

[0075] The growth of rat tumors was statistically analyzed on the presumption that it does not follow normal distribution. In the comparison between the control group and treated groups administered with respective dosages (2 group comparison), the Mann-Whitney U test was used, and a P value of 0.5 or less was judged significant. In the multiple group comparison, Scheff's F test was used, and a P value of 0.05 or less was judged significant.

(6) Results

(1) Reduction of AC-7700 toxicity with Dexamethasone

[0076] MT-9 was subcutaneously transplanted to F344 rats on their back, and after the tumor was formed, the medical agent was administered. AC-7700 was administered at the pharmaceutically effective single agent dosage of 10mg/kg, and Dexamethasone was administered at the dosage of 1mg/kg on a day before the administration of AC-7700. The biochemical indices of blood were measured 6 hours after AC-7700 administration. Results are shown in Figures 1 and 2.

[0077] The results show that Dexamethasone had remarkably reduced the toxicity of AC-7700 (10mg/kg), hepatic toxicity (GPT) and cardiovascular toxicity (CPK) in tumor bearing rats.

[0078] Concerning the gastrointestinal toxicity, the combined use of Dexamethasone with AC-7700 has revealed that diarrhea induced by AC-7700 in mice was significantly improved.

(2) Influence of Dexamethasone on the pharmaceutical effect of AC-7700

[0079] The influence of Dexamethasone on the pharmaceutical effect of AC-7700 was investigated using F344 rats, subcutaneously transplanted MT-9. AC-7700 was administered at the dosage of 10mg/kg, and 1mg/kg of Dexamethasone was administered on the day before the AC-7700 administration. Both the medical agents were 3 times admin-

istered every 3 days.

[0080] AC-7700 significantly inhibited tumor growth in a single agent. The administration of AC-7700 in combination with Dexamethasone also inhibited tumor growth in the same manner (refer to Table 1). There was no significant difference between anti-tumor effect of AC-7700 and those of AC-7700 combined with Dexamathasone (Scheffe's F test).

[Table 1]

Influence of Dexamethasone on the pharmaceutical effect of AC-7700		
DEX(mg/kg/day)	AC-7700(mg/kg/day)	I.R.(%)
0	0	0
0	10	84**
1	0	21
1	10	72**

(Note: Mann-Whitney's U test;
**: $p<0.01$ vs. Control)

[0081] Furthermore, in the investigation using CDF1 mice (female), Dexamethasone (5mg/kg/day administered by intravenous injection into the tail, on a day before and on the day of the AC-7700 administration), increased the maximum tolerable dose of AC-7700 (3 times subcutaneously administered every 3 days) from 43.6mg/kg/day to more than 90mg/kg/day.

Advantages of the Invention

[0082] According to the present invention, an anti-tumor agent containing a tubulin polymerization-inhibitory active substance as an effective component is provided, wherein the medical agent (anti-tumor agent) in combination with an anti-inflammatory active substance can retain its pharmaceutically effective dosage but can have remarkably improved toxicity of the tubulin polymerization-inhibitory active substance and an increased the lethal dosage so that its safety zone can be expanded.

[0083] The safety zone is expanded and thus its application on by medical doctors and others in the therapy, betterment, etc. of tumors, while the burden on patients and others can be reduced.

[0084] In addition, the present invention also provides the followings:

i) A method for the anti-tumor, wherein the method includes methods for a medical treatment and an improvement of tumors, a prevention of progression of tumor, and a prevention of tumor;
ii) Uses of the above-mentioned 2 effective components for a medical product such as an anti-tumor agent; and
iii) A combination of the above-mentioned 2 effective components wherein the two components are used as a medical product such as an anti-tumor agent, simultaneously or separately.

[0085] Accordingly, the present invention can be carried out in the field of medical products and the like, and therefore is very useful industrially.

Claims

1. An anti-tumor agent comprising at least a tubulin polymerization-inhibitory active substance having anti-tumor activity and at least an anti-inflammatory active substance.

2. An anti-tumor agent according to Claim 1, wherein the tubulin polymerization-inhibitory active substance is selected from the group consisting of combretastatines and derivatives thereof, vinca alkaloids and derivatives thereof, colchicinoids and derivatives thereof, dolastatins and derivatives thereof, podophyllotoxins and derivatives thereof, steganacins and derivatives thereof, amphethiniles and derivatives thereof, flavonoids and derivatives thereof, rhizoxins and derivatives thereof, curacins A and derivatives thereof, epothilones A and derivatives thereof, epothilones B and derivatives thereof, welwistatins and derivatives thereof, phenstatins and derivatives thereof, 2-stryl-quinazoline-4(3H)-ones and derivatives thereof, stilbenes and derivatives thereof, 2-aryl-1,8-naphthyridin-4(1H)-ones and derivatives thereof, 5,6-dihydroindolo(2,1-a) isoquinolines and derivatives thereof, 2,3-benzo(b)thi-

ophenes and derivatives thereof, 2,3-substituted benzo(b)furans and derivatives thereof, 2,3-substituted indoles and derivatives thereof, and 2-methoxyestradiol.

3. An anti-tumor agent according to Claim 1, wherein the anti-inflammatory active substance is selected from the group consisting of anti-inflammatory active steroid substances and analogous compounds thereof, anti-inflammatory active non-steroid substances and analogous compounds thereof, and anti-inflammatory or immuno-suppressive active substances.

4. An anti-tumor agent according to Claim 1 or 3, wherein the anti-inflammatory active substance is an anti-inflammatory active steroid substance.

5. An anti-tumor agent according to Claim 1 or 4, wherein the anti-inflammatory active substance is selected from the group consisting of Dexamethasone, prednisolone, methyl prednisolone, betamethasone, triamcinolone, paramethasone, beclomethasone, fluocinolone acetonide, cortisol, and derivatives thereof.

6. An anti-tumor agent according to Claim 5, wherein the Dexamethasone and derivatives thereof are selected from the group consisting of Dexamethasone, esters thereof and salts thereof.

7. An anti-tumor agent according to Claim 1, wherein the tubulin polymerization-inhibitory active substance is selected from the group consisting of combretastines and derivatives thereof and stilbenes and derivatives thereof, and the anti-inflammatory active substance is selected from the group consisting of Dexamethasone and derivatives thereof.

8. An anti-tumor agent according to Claim 1, wherein the tubulin polymerization-inhibitory active substance is (Z) -N-[2-methoxy-5-[2-(3,4,5-trimethoxyphenyl)vinyl]phenyl]-L-serinamide or salt thereof.

9. An anti-tumor agent according to Claim 1, wherein the tubulin polymerization-inhibitory active substance is in the form of an anti-tumor pharmaceutical preparation and the anti-inflammatory active substance is in the form of an anti-inflammatory agent.

10. An anti-tumor agent according to Claim 9, wherein the anti-tumor pharmaceutical preparation and the anti-inflammatory agent are separately administered.

11. An anti-tumor pharmaceutical preparation containing a tubulin polymerization-inhibitory active substance having anti-tumor activity, wherein the anti-tumor pharmaceutical preparation is to be used in combination with an anti-inflammatory active substance.

12. A toxicity-reducing agent to be used for an anti-tumor pharmaceutical preparation containing a tubulin polymerization-inhibitory active substance, wherein the toxicity-reducing agent comprises an anti-inflammatory active substance.

13. A method for the anti-tumor, which comprises administering a tubulin polymerization-inhibitory active substance having anti-tumor activity and an anti-inflammatory active substance to a living subject.

14. A method according to Claim 13, wherein the administration form is in the form of the anti-tumor agent or the anti-tumor pharmaceutical agent according to any one of Claims 1 to 11.

15. Uses of a tubulin polymerization-inhibitory active substance having anti-tumor activity and an anti-inflammatory active substance for a medical product.

16. Uses according to Claim 15, wherein the tubulin polymerization-inhibitory active substance and the anti-inflammatory active substance are separate each other in the form of pharmaceutical preparation.

17. Uses according to Claim 15 or 16, wherein the form in use for the medical product is in the form of anti-tumor agent or anti-tumor pharmaceutical preparation according to any one of Claims 1 to 11.

18. A combination of a tubulin polymerization-inhibitory active substance having anti-tumor activity with an anti-inflammatory active substance, wherein the two substances are used as a medical product simultaneously or separately.

Fig.1

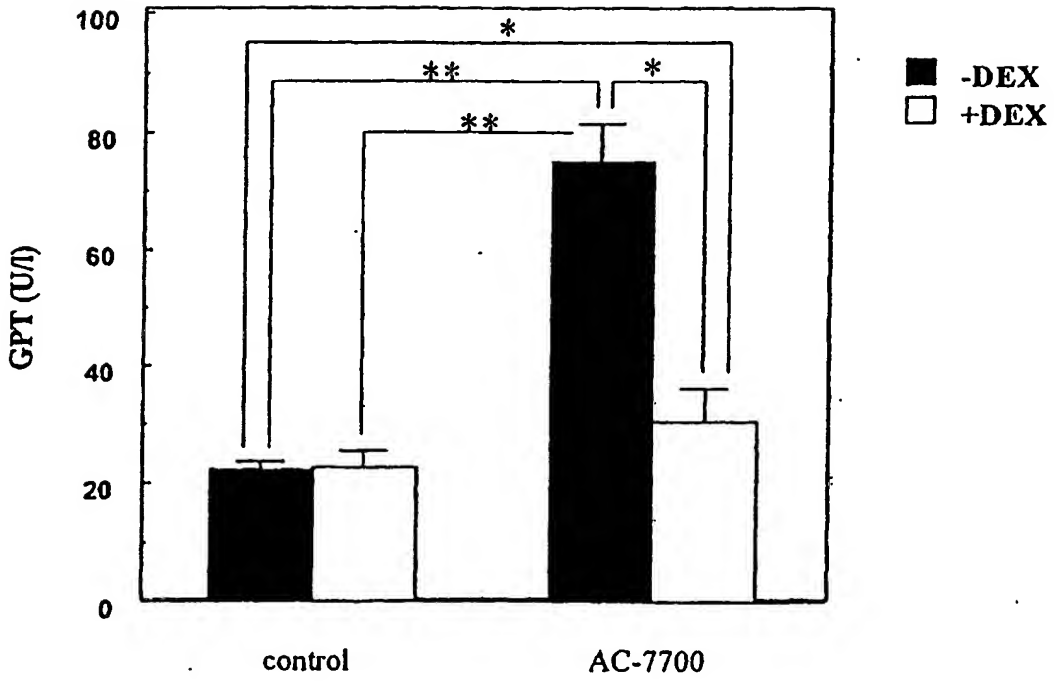
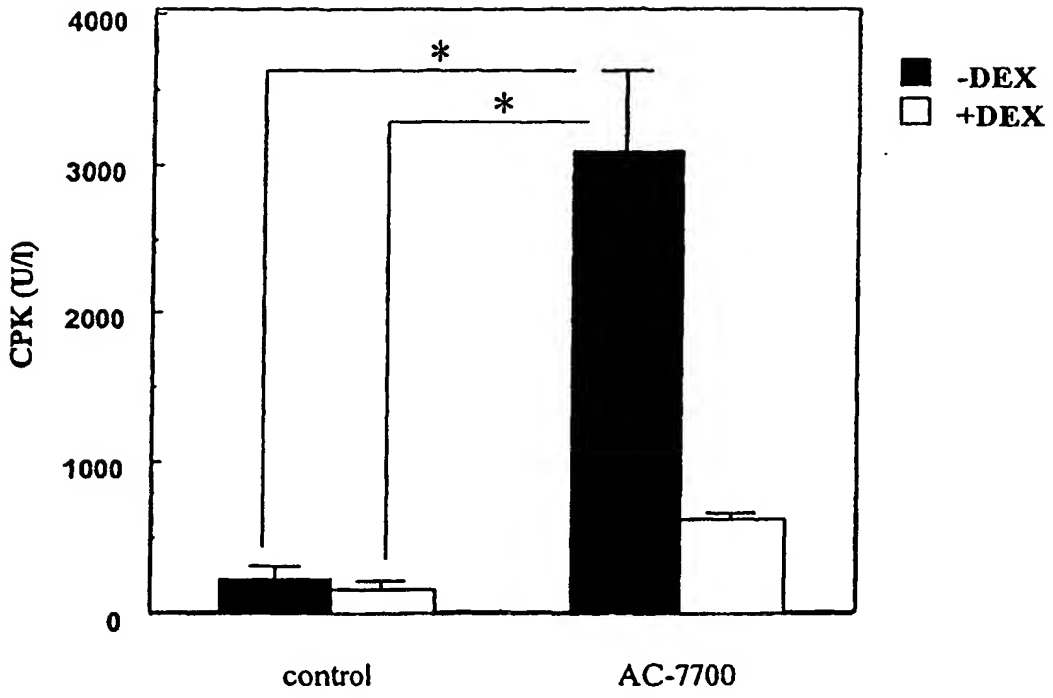


Fig.2



INTERNATIONAL SEARCH REPORT

International application No.
PCT/JP02/06260

A. CLASSIFICATION OF SUBJECT MATTER
Int.Cl[7] A61K45/08, A61P35/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
Int.Cl[7] A61K45/08, A61P35/00

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
CA(STN), MEDLINE(STN)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X Y	WO 98/32440 A1 (The Procter & Gamble Co.), 30 July, 1998 (30.07.98), Full text; particularly, page 6, lines 25 to 33 & AU 729099 B & EP 967977 A1 & CN 1244123 A & US 6329355 B1 & US 6271217 B1 & JP 2001-509164 A	1,3-6,9-12, 18 2,7-8
X Y	WO 97/05870 A2 (The Procter & Gamble Co.), 20 February, 1997 (20.02.97), Full text; particularly, page 5, lines 10 to 24 & AU 713031 B & EP 841914 A2 & JP 11-511136 A & KR 99036137 A	1,3-6,9-12, 18 2,7-8

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier document but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
06 September, 2002 (06.09.02)	17 September, 2002 (17.09.02)
Name and mailing address of the ISA/ Japanese Patent Office Facsimile No.	Authorized officer Telephone No.

Form PCT/ISA/210 (second sheet) (July 1998)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/JP02/06260

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 99/51246 A (Ajinomoto Co., Inc.), 14 October, 1999 (14.10.99), Full text & AU 747599 B & EP 1068870 A1 & CN 1303294 A & KR 2001042399 A	2,7-8
X	Raymond B. et al., Hypersensitivity Reactions From Taxol, Journal of Clinical Oncology (1990), Vol.8, No.7, pages 1263 to 1268	1,3-6,9-12, 18
X	Eckhardt S. et al., The effect of docetaxel on malignant tumos, Orv. Hetil. (1998), Vol.139, No.15, pages 867 to 872	1,3-6,9-12, 18
X	George P. Browman et al., Modified adriamycin-vincristine-dexamethasone(m-VAD) in primary refractory and relapsed plasma cell myeloma: an NCI (Canada) pilot study, British Journal of Haematology (1992), Vol.82, pages 555 to 559	1,3-6,9-12, 18

Form PCT/ISA/210 (continuation of second sheet) (July 1998)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/JP02/06260

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [x] Claims Nos.: 13-17

because they relate to subject matter not required to be searched by this Authority, namely:

Claims 13 to 17 pertains to methods for treatment of the human body by therapy and thus relates to a subject matter which this International Searching Authority is not required, under the provisions of Article 17(2)(a)(i) of the PCT and Rule 39.1(iv) of the Regulations under the PCT, to search.

2. [ ] Claims Nos.:

because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. [ ] Claims Nos.:

because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest
[ ] The additional search fees were accompanied by the applicant's protest.
[ ] No protest accompanied the payment of additional search fees.

Form PCT/ISA/210 (continuation of first sheet (1)) (July 1998)